

35. The Early Development of the Heart and Anterior Vessels in Marsupials, with Special Reference to *Perameles*. By KATHARINE M. PARKER, B.Sc. (Lond.), Assistant in the Department of Zoology, University of London, University College*.

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(Plates I., II.† and Text-figures 1-25.)

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INTRODUCTION.

The following piece of work was undertaken at the suggestion of Professor J. P. Hill, for whose invaluable help in carrying it out I am deeply grateful. The embryos studied are all from his collection and text-figs. 1 and 6 were made from photographs taken by him.

I wish also to thank Miss E. A. Steele for her beautiful figures of the model (Stage V.) and Mr. F. C. Pittock for much help in making the model.

The material affords an excellent opportunity for the study of the mode of development of the pleuro-pericardial canals, the origin and differentiation of the endothelial heart-tubes, and the method of fusion of the lateral primordia of the heart, and it was in the hope of making some progress towards the solution of the interesting problems of early cardiac development that the work was undertaken.

The general arrangement of the pericardium, heart, and aorta in the early stages (viz. Stages I. to IV. inclusive) can be accurately determined by graphic reconstruction. Figures 1 to 5 (Pl. I.) were all obtained by this method, and are intended to give some idea of the relations of the pericardium and heart to the gut and the brain-plate in successive stages.

In the next stage (Stage V.) the curvature of the heart makes it impossible to represent the relations of the parts accurately in two dimensions. A wax-plate reconstruction was therefore made (see Pl. II.), and as all the vessels of the head which were

* Communicated by Prof. J. P. HILL, D.Sc., F.R.S., F.Z.S.

† For explanation of the Plates see p. 499.

recognisable as such were included in the model, a certain amount of light was shed on the development and early relations of the cardinal veins and aortic arches. The following account therefore deals not only with the development of the heart, but also with certain facts relative to the early development of the vessels of the head.

DESCRIPTION OF STAGES.

STAGE I. *Perameles obesula* (1 Z).
Dasyurus viverrinus (7.5 mm. vesicle).

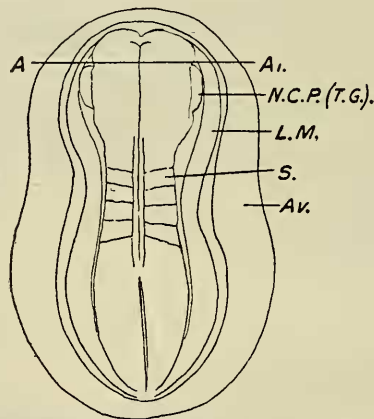
The material for this stage consists of four embryos of *Perameles obesula*, one cut longitudinally and three transversely, and several of *D. viverrinus*.

(a) *Perameles obesula* (1 Z).

* Total length of embryo A in curved condition = 6.08 mm.

In this stage there is a flat brain-plate with an extensive neural crest proliferation in the cranial region (text-fig. 1, *N.C.P. (T.G.)*).

Text-figure 1.



Perameles obesula (1 Z). Dorsal view of embryo A.

Aκ. Area vasculosa. *L.M.* Lateral mesoderm. *N.C.P. (T.G.)*. Neural crest proliferation (primordium of trigeminal ganglion). *S.* Somite. *A, A1*. Level of section represented in text-fig. 2.

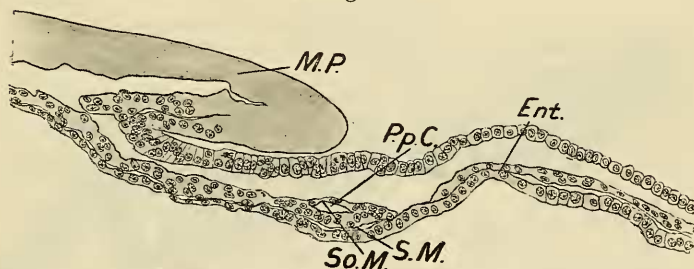
There are four distinct somites (*S.*): behind the fourth is a somitic thickening with an indistinct posterior limit, and in front of the first, another imperfectly defined mass, representing presumably a transitory first somite.

* All measurements of embryos and certain descriptive details are taken from Professor Hill's original notes and photographs of the material.

Laterally to the somitic mesoderm and the medullary plate is a zone appearing clear in surface view, in which the mesoderm forms a thin sheet. Outside this again is an opaque zone of lateral mesoderm (*L.M.*), completely surrounding the embryo. This is bounded peripherally by a clear zone which separates it from the area vasculosa (*Av.*). The entoderm forms a thin continuous layer, while a small incipient head-fold definitely marks the anterior margin of the brain-plate and involves also the protochordal plate lying in the middle line immediately below it.

In the lateral mesoderm on each side a horizontal cleft has appeared, separating the mesoderm into a dorsal somatic and a ventral splanchnic layer. These clefts, commencing on both sides of the embryo and extending forwards, constitute the pleuro-pericardial canals, the form of which can readily be seen in Pl. I. fig. 1 (*P.p.C.*) The canals form a horseshoe the median

Text-figure 2.



Perameles obesula (1Z, B). Transverse section in plane A, A₁.
(See text-fig. 1.)

Ent. Entoderm. *M.P.* Medullary plate. *P.p.C.* Pleuro-pericardial canal.
S.M. Splanchnic mesoderm. *So.M.* Somatic mesoderm.

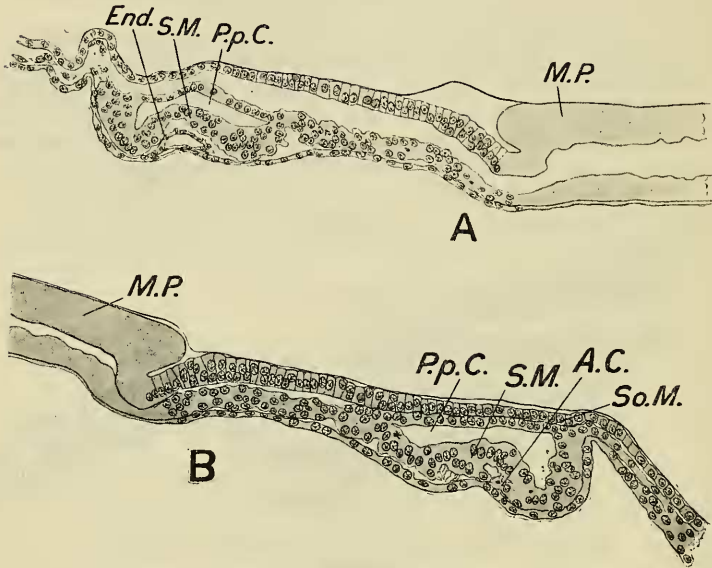
anterior portion of which lies underneath the anterior margin of the brain-plate (*M.M.P.*), while the lateral limbs extend back into the somitic region. In the anterior region, the dorso-ventral extent of the pleuro-pericardial canal is very small (text-fig. 2, *P.p.C.*), and in fact the continuity of the canal is actually interrupted on the right side of the embryo (Pl. I. fig. 1).

The cleft increases markedly in size in the region of the trigeminal neural crest proliferation (text-fig. 1, *N.C.P. (T.G.)*) and attains its greatest size in the hind-brain region (text-fig. 3 A & B). Behind this it becomes reduced in size until, opposite the posterior somites, the coelomic cavity is represented by irregular clefts in the lateral mesoderm.

The pleuro-pericardial canals throughout their extent have a thin somatic and a slightly thicker splanchnic wall (see text-figs. 2, 3 A & B, *So.M.*, *S.M.*), which in the anterior region is in close

contact with the entoderm (text-fig. 2). In the region of the maximum size and development of the pleuro-pericardial canals (Pl. I. fig. 1) the endothelial primordia of the heart are differentiating (text-fig. 3, *End.*) between the entoderm and the splanchnic mesoderm, which therefore projects as a prominent fold into the pleuro-pericardial canal.

Text-figure 3.

*Perameles obesula* (1Z, B).

A. Transverse section in region of greatest width of pleuro-pericardial canals, with endothelial tubes developed. B. Transverse section showing origin of angioblast cells from the splanchnic mesoderm.

A.C. Angioblast cell. *End.* Endothelium. *M.P.* Medullary plate. *P.p.C.* Pleuro-pericardial canal. *S.M.* Splanchnic mesoderm. *So.M.* Somatic mesoderm.

The endothelial heart primordia are best developed in their posterior portions, where they are actually tubular in some embryos of this stage (see text-fig. 3 A). Anterior to the tubular portion, the primordia are represented by solid cords of angioblast cells, isolated examples of which are found scattered along the length of the pleuro-pericardial canals in the positions indicated in Pl. I. fig. 1 (*A.C.*). It may be concluded from this that the endothelial tubes differentiate postero-anteriorly, and that their increase in length is brought about not by direct forward growth of the first formed parts of the tubes, but by the

progressive differentiation of angioblast cells in the cephalic portions of the pleuro-pericardial canals.

The evidence of this stage does not justify any definite statement with regard to the origin of the endothelium of the heart. From text-fig. 3 B it will be seen that the splanchnic mesoderm (*S.M.*) shows distinct traces of proliferative activity; its ventral, indented margin has an irregular outline and there are indications of loosening of the cells. On the other hand, there is no definite evidence of entodermal proliferation, though in an earlier stage, which will form the subject of a separate paper, the appearances by no means exclude the possibility of the entodermal origin of the endothelium, whilst there is clear evidence of proliferative activity on the part of the entoderm of the area vasculosa.

(b) *Dasyurus viverrinus* (7.5 mm. vesicle).

The material on which the following description is based consists of two embryos, one cut transversely, the other longitudinally.

Greatest length of each embryo = 7 mm.

In this stage there is a flat brain-plate, the anterior margin of which is marked in the middle line by a thickened terminal ridge (text-fig. 4, *T.R.*). There is a well developed neural crest proliferation, the anterior portion representing the primordium of the trigeminal ganglion, and the posterior that of the facial, glosso-pharyngeal and vagus ganglia. No somites are yet differentiated.

The outline of the anterior end of the brain-plate and the pleuro-pericardial canals and endothelial heart-tubes are shown in Pl. I. fig. 2. From this it will be seen that the pleuro-pericardial canals (*P.p.C.*) extend continuously round the head-end of the embryo and lie anterior to the anterior margin of the brain-plate (*M.M.P.*).

The pleuro-pericardial canals attain their greatest size in the hind-brain region (*H.B.*) at this stage, and here also the primordia of the heart are well established in the form of endothelial tubes lying between the entoderm and the thickened splanchnic mesoderm. The endothelial tubes terminate anteriorly at the level of the posterior limit of the trigeminal primordium. In front of this, however, there are scattered angioblast cells and strands of cells extending forwards as indicated in Pl. I. fig. 2. In the condition of the heart primordia the *Dasyurus* embryos are in advance of those of *Perameles* described above, for the endothelium is definitely tubular throughout a great portion of its extent and the myocardial fold is consequently well developed.

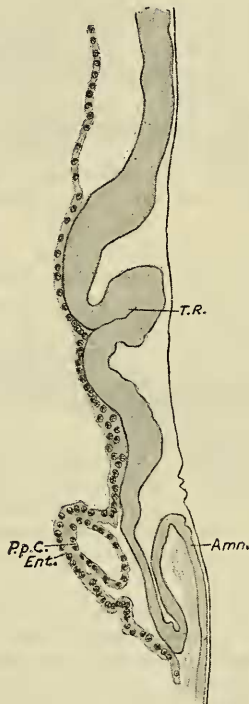
From the longitudinal section (text-fig. 4) it will be seen that the antero-median portion of the pleuro-pericardial canal lies some distance in front of the anterior margin of the brain-plate (*T.R.*).

In Stage I., then, we have a horseshoe-shaped pleuro-pericardial

cavity extending round the embryo and with the lateral limbs prolonged into the somitic region. The endothelial primordia of the heart are represented by more or less continuous tubes, solid cords, and scattered angioblast cells differentiating in the postero-anterior direction and lying between the entoderm and the splanchnic mesoderm.

The endothelial heart-tubes are the only vessels yet established.

Text-figure 4.



Dasyurus viverrinus (7.5 mm. vesicle). Longitudinal section, median through the anterior margin of the brain-plate.

Amn. Head-fold of amnion. *Ent.* Entoderm. *P.p.C.* Pleuro-pericardial canal. *T.R.* Terminal ridge.

STAGE II. *Dasyurus viverrinus* (8.5 mm.).

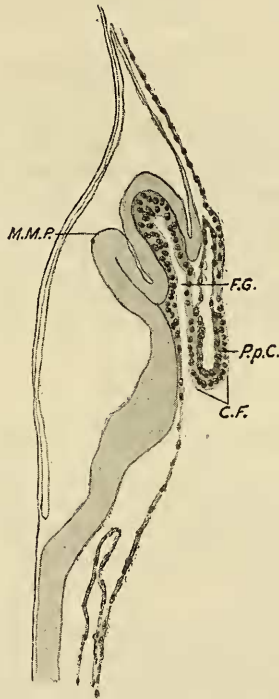
The material for this stage consists of one embryo (A) of *Dasyurus viverrinus* cut transversely and one (A α) cut longitudinally. A graphic reconstruction of the gut and pericardium together with the endothelial heart-tube and aorta of the left side of embryo A will be found in Pl. I. fig. 3.

Total length of embryo A, 8.5 mm.

This embryo possesses a flat brain-plate with well marked optic grooves; somites are not as yet distinctly differentiated.

The position of the anterior margin of the brain-plate is indicated in fig. 3 (*M.M.P.*) as well as the outline of the gut (*F.G.*). On comparison with fig. 2, it is clear that the progress of the head-fold has brought about considerable alteration in the

Text-figure 5.



Dasyurus viverrinus (8.5 mm.). Longitudinal section of embryo A α , median through anterior end.

C.F. Cardiac fold. *F.G.* Fore-gut. *M.M.P.* Margin of medullary plate.
P.p.C. Pleuro-pericardial canal.

relations of the gut, brain, and pericardium (see Pl. I. fig. 3 and text-fig. 5). In embryo A (fig. 3), which is slightly in advance of embryo A α (text-fig. 5), the anterior margin of the brain-plate now marks the actual anterior limit of the embryo, while at the same time the crescent-shaped fore-gut (*F.G.*) has developed. Its anterior limit lies immediately behind the anterior margin of the brain-plate, while closely applied to its posterior and lateral

margins lies the continuous pericardial cavity which shows a marked increase in size as compared with the previous stage. (Compare Pl. I. figs. 2 & 3, *P.p.C.*) This increase is greater in the median anterior limb of the pericardium than in its lateral portions. (Compare text-figs. 4 & 5.)

The differentiation of the endothelial primordia of the heart has progressed considerably and they now extend as actual tubes even to the middle line of the pericardium. This fact is somewhat remarkable, as such a condition, in which the lateral heart-tubes are actually in contact at their extreme cephalic apices and diverge widely and abruptly from this point of contact, is not found in any other stage.

Some distance behind its cephalic extremity, the lateral heart-tube gives rise to the first aortic arch (fig. 3, *A 1*), which follows the antero-lateral margin of the gut almost to the middle line and there becomes continuous with the corresponding dorsal aorta, the two aortæ being completely established in this stage.

It will be unnecessary to go into further details of the structure of this embryo, as the sectional appearances found in it are exemplified yet more clearly in the *Perameles* embryo to be described next. It is, however, an important stage with regard to the processes of growth and folding which bring about the relations of the brain, gut, and pericardium which are found in subsequent stages.

STAGE III. *Perameles nasuta* (1 S).

The material in this stage consists of two flat embryos with widely open brain-plates and lateral heart-tubes. Embryo A has eleven somites, the first being small and indistinctly limited; embryo B has nine, the most anterior being indistinct here also. The following description is based mainly on embryo A, which was cut transversely.

Total length of embryo A after partial flattening under cover-glass, from the anterior margin of the brain-plate to the hinder extremity of the primitive streak : 7.5 mm.

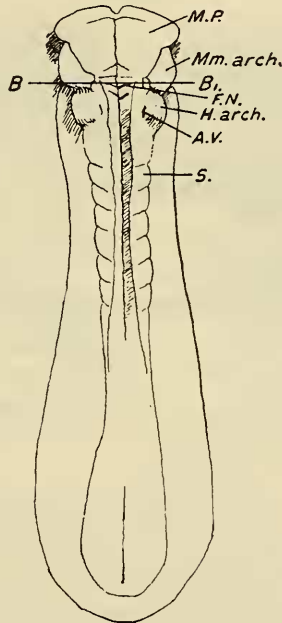
Vascular area, 8.96×5.8 mm. in diameter.

Text-fig. 6 represents a dorsal view of embryo A, while the outlines of the gut and pericardium and the endothelial heart-tube and aorta of the left side are given on Pl. I. fig. 4. From these figures it will be seen that the stage shows a considerable advance in general development on the preceding. The head-fold has progressed back as far as the region of the auditory pit. The brain, though flat and widely open in the fore- and mid-brain regions, is deeply grooved in the hind-brain region. Lying lateral to the brain-plate are two pairs of mesodermal masses, one the maxillo-mandibular process, the other the hyoid arch. Between these two arches is the first visceral pouch, and behind the hyoid arch the second visceral pouch is already developed.

Shortly behind this, the paraxial mesoderm is differentiated into somites.

The maxillo-mandibular process (text-fig. 6, *Mm. arch.*) forms a dense mass of mesoderm not distinctly marked out into maxillary and mandibular portions. It lies laterally to the gut on the outer side of the lateral and dorso- and ventro-lateral walls of the gut, and extends antero-posteriorly from almost the anterior end of the gut back to the first visceral pouch, which is situated level with the anterior intestinal portal. The entoderm of the first visceral pouch reaches the ectoderm, but the closing membrane is not perforated.

Text-figure 6.



Perameles nasuta. Dorsal view of embryo A.

B, B₁ indicates the level of the section represented in text-fig. 7.

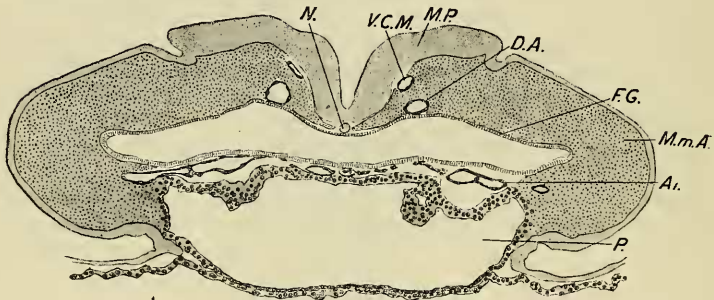
A.v. Auditory vesicle. *F.N.* Facial neuromere. *H.arch.* Hyoid arch. *Mm. arch.* Maxillo-mandibular arch. *M.P.* Medullary plate. *S.* Somite.

The hyoid arch is a mass of mesoderm only slightly smaller in surface view than the maxillo-mandibular arch, but situated entirely dorsal to the gut. Behind it is the second visceral pouch, which is small and does not reach the ectoderm (Pl. I. fig. 4, *V.P.* 2).

The form of the pericardium and its relations to the gut,

as well as the topography of the endothelial heart-tubes and the aortæ and aortic arches, will be understood best by reference to fig. 4 and text-fig. 12. From fig. 4 it will be seen that the pericardium (*P.*) has increased markedly in size. As in the previous stage, its inner wall is closely applied to the entoderm of the crescentic or U-shaped anterior intestinal portion (*A.I.P.*) (cf. text-fig. 12). The antero-median portion of the pericardium is somewhat rectangular in transverse section (text-fig. 7); its dorsal wall is slightly thicker than its ventral, and between the former and the floor of the gut lie the endothelial heart-tubes (text-fig. 8) and the first aortic arches (text-fig. 7).

Text-figure 7.



Perameles nasuta (1 S, A.). Transverse section through median pericardium and first aortic arch.

*A*₁. First aortic arch. *D.A.* Dorsal aorta. *FG.* Fore-gut. *M.P.* Medullary plate. *M.m.A.* Maxillo-mandibular arch. *N.* Notochord. *P.* Pericardium. *V.C.M.* Vena capitis medialis.

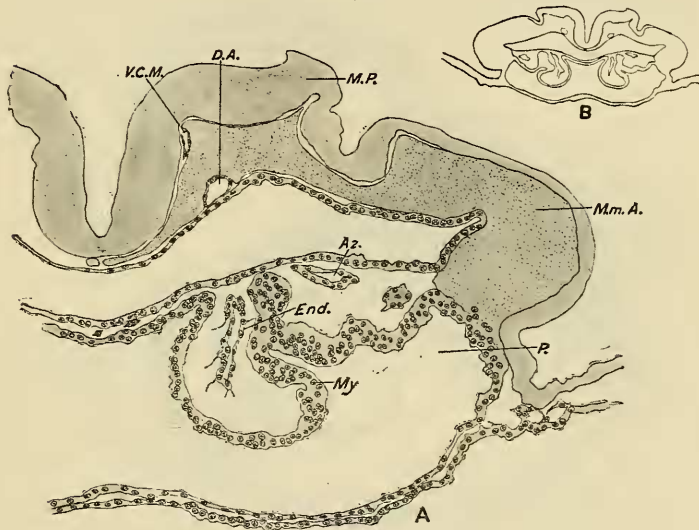
In the antero-median limb of the pericardium, the endothelial heart-tubes, enclosed in a fold of splanchnic mesoderm, lie separate from each other below the closed gut (Pl. I. fig. 4 and text-fig. 8), while at the level of the anterior intestinal portal they diverge in the manner indicated in fig. 4 and lie on either side of the open gut in the dorso-medial wall of the pericardium (text-fig. 9). In their posterior portions the heart primordia lie on the ventral side of the pericardium.

The endothelial heart-tubes throughout their length are almost completely enclosed by the layer of splanchnic mesoderm constituting the primordium of the myocardium. In the posterior region of the heart the myocardial layer is closely applied to the outside of the endothelial tube (text-fig. 10), but in the greater part of its extent there is a considerable space between the two layers of the heart primordium (text-fig. 9).

From the cephalic extremity of each endothelial tube, there arise two vessels, one of which runs forwards and outwards

towards the lateral margin of the gut and then parallel with this margin (Pl. I. fig. 4, A 1). It loops round the anterior limit of the gut, joins the dorsal aorta, and thus constitutes the first or mandibular aortic arch. It is impossible here to fix any exact limit between the endothelial heart-tube and the aortic arch. Text-figs. 7 and 8 represent typical sections through each. The transition from the heart primordium to the aortic arch is indicated by the gradual reduction in the dorsi-ventral extent of the space surrounding the endothelial tube and the rotation of the vessel, so that its greatest diameter comes to lie parallel

Text-figure 8.

*Perameles nasuta* (1S, A.).

A. Transverse section through heart primordia and second aortic arch.

B. Complete outline of same section.

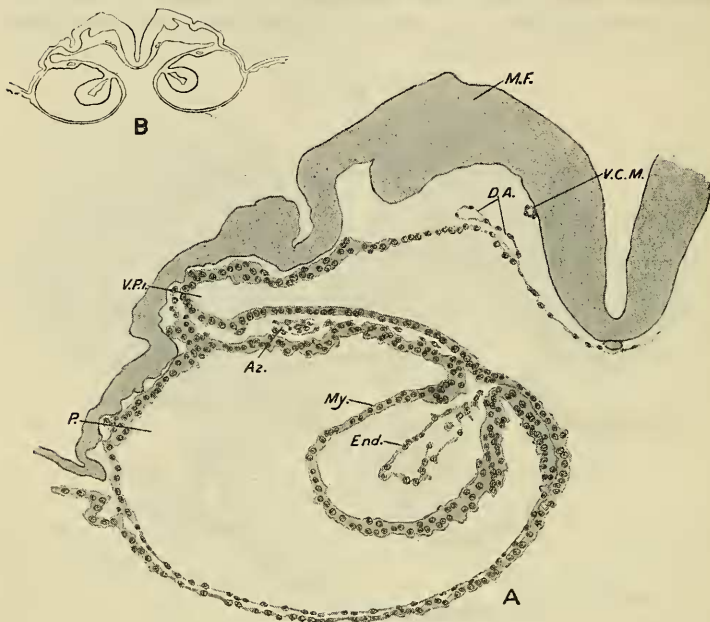
- A₂. Second aortic arch. D.A. Dorsal aorta. End. Endothelium of heart.
 M.m.A. Maxillo-mandibular arch. M.P. Medullary plate. My. Myocardium.
 P. Pericardium. V.C.M. Vena capitis medialis.

with the floor of the gut. (Compare text-figs. 8 and 7.) Outside the limits of the pericardium the aortic arch runs in the mesoderm of the maxillo-mandibular process (text-fig. 7). It is connected with the dorsal aorta by one main loop and by several smaller vessels which were omitted from fig. 4 for the sake of clearness.

The second vessel, which arises from the anterior end of the heart, is small and runs backwards and outwards, lateral to and almost parallel with the heart-tube, and has precisely the same relations to the gut and mesoderm.

Comparison with the succeeding stages shows that this represents the ventral portion of the future second aortic arch (Pls. I., II., figs. 4 & 8, A 2), while a small vessel arising from the dorsal aorta and running outwards on the dorsal wall of the gut ventral to the auditory vesicle, corresponds with the dorsal portion of the completed arch in later stages.

Text-figure 9.

*Perameles nasuta* (1 S, A.).

A. Transverse section in region of lateral heart primordia and open gut.
B. Complete outline of same section.

A₂. Second aortic arch. D.A. Dorsal aorta. End. Endothelium of heart.
M.F. Medullary fold. My. Myocardial layer. P. Pericardium. V.C.M. Vena
capitis medialis. V.P.1. First visceral pouch.

In the median space between the anterior ends of the endothelial heart-tubes are a number of scattered angioblast cells lying between the splanchnic mesoderm and the entoderm (Pl. I. fig. 4 and text-fig. 7). These cells possibly represent the primordia of the capillaries found in the corresponding position in the next stage. They afford an instance of the origin of angioblast cells from the splanchnic mesoderm after the establishment of the definitive endothelial heart-tubes.

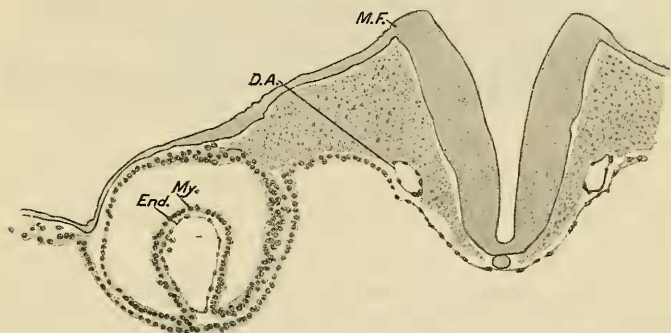
Posterior to the region represented in fig. 4, the heart-tubes

gradually curve outwards and pass imperceptibly into vitelline veins.

The dorsal aorta is paired and runs back continuously, the two vessels keeping approximately the same distance from the middle line (Pl. I. fig. 4, *D.A.*).

Immediately dorsal to the dorsal aorta on each side there is situated a series of apparently isolated sections of a minute blood-vessel (text-fig. 7, *V.C.M.*). These capillaries lie close against the medullary tube, medially to the neural crest proliferation in the region of the trigeminal ganglionic primordium. From the position of this vessel relative to the dorsal aorta and nerves, it is evidently the vena capitis medialis of Grosser (5). A brief summary of some of the literature on the subject of the venæ capitis medialis and lateralis and their relations to the anterior cardinals will be found below, together with a review of the facts of development of these veins in *Perameles*.

Text-figure 10.



Perameles nasuta (1 S. A.). Transverse section through posterior portion of lateral heart primordia.

D.A. Dorsal aorta. *End.* Endothelium of heart. *M.F.* Medullary fold.
My. Myocardial layer.

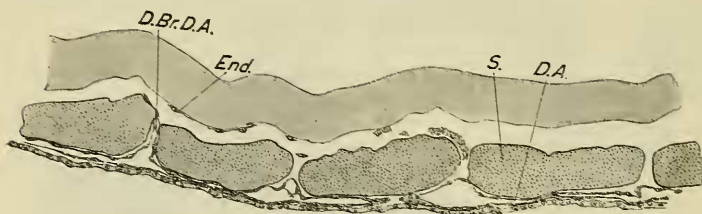
Although it is not within the scope of this paper to deal in any detail with the posterior vessels, it may be noted here that in this stage, in the region of the posterior somites, there occur intersegmental offshoots from the dorsal aortæ. These intersegmental offshoots are shown very clearly in the longitudinal series (text-fig. 11). It will be seen from the figure that between each two successive somites (*S.*) there is a small dorsal offshoot from the aorta. Dorsal to the somites there are a few scattered endothelial cells (*End.*). The longitudinal vessel connecting the offshoots is not continuous, but portions of it are present in the next section.

At the level of the second somite there occurs a pair of small vessels lying in the somatopleure immediately dorsal to the heart-tubes. Each consists of a single vessel with a few minute

branches and is blind at both ends and not connected as yet with any other capillaries. From these vessels the Cuvierian ducts are later developed.

In this stage we have, therefore, lateral heart-tubes which, while they approach one another anteriorly, are widely separate in the posterior region. Paired dorsal aortae and the first pair of aortic arches are developed, whilst traces of the second arch are also present. This stage accordingly agrees in its general features with the $8\frac{1}{2}$ days rabbit described by Bremer (1). In addition to the heart and arterial vessels there are also present in the head the first traces of the venous system in the form of disconnected portions of the vena capitis medialis and the primordia of the Cuvierian ducts.

Text-figure 11.



Perameles nasuta (1 S, B.). Longitudinal section through somites showing dorsal offshoots from the dorsal aorta.

D.A. Dorsal aorta. D.Br.D.A. Dorsal branch of same. End. Endothelium of longitudinal vessel. S. Somite.

STAGE IV. *Perameles nasuta* (2 P).

The material consists of four embryos, A and C cut transversely, B and D longitudinally.

Each of the four embryos has fifteen or sixteen somites. The neural tube is still unclosed throughout its length, but the folds are closely approximated in the hind-brain region. The mid- and fore-brain segments are widely open as in Stage III., but the primary cranial flexure has occurred so that the fore-brain is bent forwards and downwards (see text-fig. 15).

The relations of the gut, pericardium, and heart are indicated in Pl. I. fig. 5. It is well to note at this point that the outline of the brain-plate as indicated in figure 5 is not strictly comparable with the corresponding line in fig. 4 (compare text-figs. 12 and 15). The fact that the primary cranial flexure has occurred, renders it impossible to plot the morphological anterior end of the brain in the same plane reconstruction with the hind-brain, gut, etc. The difference in the relations of the brain to the pericardium in the two stages may, however, be judged by the position of the auditory neuromere (A.N.), which lies at the posterior margin of the pericardium in

Stage, III. and at the anterior margin thereof in the present stage.

The first and second visceral pouches are now well marked (fig. 5, *V.P.* 1 & 2), while the relations of the maxillo-mandibular process and hyoid arch show little advance on the preceding stage.

The antero-median portion of the pericardium has increased very considerably in the antero-posterior direction. Furthermore, it may be noted that as the portion of the gut lying anterior to the first visceral pouch has remained the same length (compare figs. 4 & 5) and the anterior margin of the pericardium is now situated in the same plane with the first visceral pouch, the pericardium must have moved backwards as a whole.

Text-figure 12.



Perameles nasuta (1S, B). Longitudinal section, median through the anterior end.

A.I.P. Anterior intestinal portal. *C.F.* Cardiac fold. *M.M.P.* Margin of medullary plate. *P.* Pericardium. *P.P.* Protochordal plate.

The form of the endothelial primordia of the heart is shown in fig. 5. They have fused at their cephalic extremity, the fused portion extending through some eighteen sections and representing the most closely approximating portions of the endothelial tubes in Stage III. (Pl. I. fig. 4). From it is derived the bulbus (conus) arteriosus of the next stage (Pl. II. fig. 8, *B.A.*). Posterior to this fused portion, the endothelial tubes lie close together but unfused for a considerable portion of their length (Pl. I. fig. 5 and text-fig. 14), and then diverge widely and pass into vitelline veins. The endothelial tubes throughout their length are enclosed by the myocardial wall, which shows characteristic thickening and prolongations of its cells throughout the greater part of the length of the heart (text-fig. 14, *My.*).

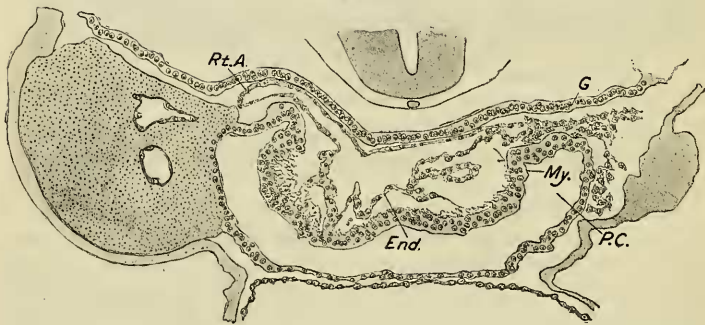
The myocardium of the right and left sides is united from the cephalic extremity of the heart primordium to the point of divergence of the right and left endothelial tubes, but the line of fusion is not marked by any groove. In the posterior region where the endothelial tubes separate from each other, each is surrounded by its own myocardial layer, so that for a short distance in front of the anterior intestinal portal, the two heart-tubes lie below the closed gut, each surrounded by an independent fold of splanchnic mesoderm. The primordia of the heart

are prolonged into the lateral gut-folds and pass gradually into vitelline veins.

The heart as a whole is somewhat asymmetrical (see fig. 5), being curved over to the right side of the embryo.

The aortic arches, two of which are developed, arise from the median bulbus arteriosus. The endothelial heart-tube bifurcates in front, and each half runs forwards and slightly outwards as a relatively wide vessel situated between the two layers of the splanchnopleure. These vessels, which constitute the first aortic arches, run forwards and outwards in a course similar to that of the same vessels in the next stage (compare figs. 5 and 6). Anteriorly they loop round the gut to join the dorsal aortæ. From the anterior convexity of this first aortic arch are given off capillaries which form a network surrounding the primary optic vesicles. From the lateral margin of the fused tip of the heart is given off on each side a small vessel which runs outwards and backwards, loops round the gut, and constitutes a continuous though slender second aortic arch.

Text-figure 13.



Perameles nasuta (2 P, A). Transverse section through bulbus arteriosus.

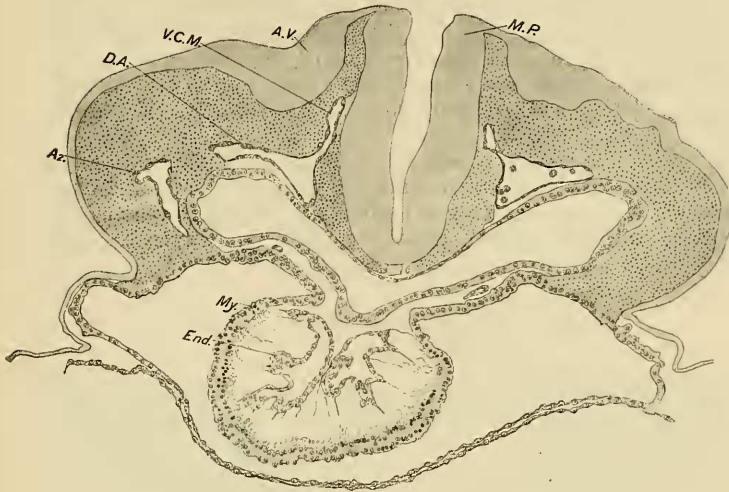
End. Endothelium. G. Gut. My. Myocardium. P.C. Pericardial cavity.

Rt.A. Root of aortic arch.

The vena capitis medialis, which was just recognisable in the preceding stage, is now considerably further developed. It is represented by an irregular and not perfectly continuous series of capillaries, situated dorsal to the aorta on either side of the medullary tube, medial to the cranial ganglia. These capillaries are connected by very fine sprouts with the dorsal aortæ (text-fig. 14, *V.C.M.*); ventro-lateral to the auditory vesicle and lateral to the nerve-roots, there is another line of scattered capillaries connected with the vena capitis medialis. These are the first traces of the vena capitis lateralis. Portions of the vena capitis medialis can be traced in the region of the lateral heart-tubes as far back as the Cuvierian ducts (*i. e.*, the region of the third

somite). Behind this again there are, as in Stage III., intersegmental offshoots from the dorsal aorta, but as yet no continuous vessel in this region.

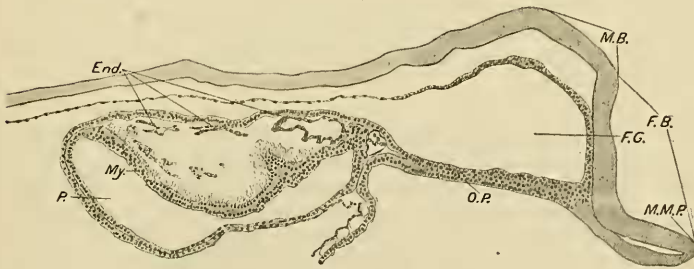
Text-figure 14.



Perameles nasuta (2 P, A). Transverse section through ventricular region of the heart.

*A*₂. Second aortic arch. *A.V.* Auditory vesicle. *D.A.* Dorsal aorta. *End.* Endothelium. *M.P.* Medullary plate. *My.* Myocardium. *V.C.M.* Vena capitis medialis.

Text-figure 15.



Perameles nasuta (2 P, B).

Longitudinal section, median through the anterior end of the embryo.

End. Endothelium. *F.B.* Fore-brain. *F.G.* Fore-gut. *M.B.* Mid-brain. *M.M.P.* Margin of medullary plate. *My.* Myocardium. *O.P.* Oral plate. *P.* Pericardium.

In this stage the Cuvierian ducts are recognisable lying in the somatopleure opposite the third somite and immediately
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dorsal to the heart-tube. The main trunk of each ends blindly anteriorly and posteriorly and is of considerable size, causing a bulging of the mesoderm of the somatopleure, which is thus brought into contact with the mesoderm surrounding the heart-tube. As yet, however, neither of the Cuvierian ducts opens into the heart-tube. From the medial side of each Cuvierian duct a few small capillaries are given off. They run towards the middle line and represent that portion of the anterior cardinal vein which at a later stage connects the venæ capitis medialis and lateralis with the Cuvierian duct. (Compare Pl. II. fig. 7, *A.C.V.*)

Stage IV., therefore, possesses a heart in which the endothelial tubes have fused anteriorly and curvature has already commenced. Two complete aortic arches, an incomplete vena capitis medialis, traces of a vena capitis lateralis, and Cuvierian ducts are present.

STAGE V. *Perameles obesula* (10. viii. 03).

Macropus sp.

The material for this stage consists of five similar embryos of *P. obesula*, three cut transversely and two longitudinally, and one embryo of *Onychogale frenata* (? *Macropus* sp.) cut transversely. In several respects, *e. g.* curvature of the heart, the *Onychogale* embryo represents a slightly earlier stage than *Perameles obesula* (10. viii. 03). For purposes of description, however, it will be convenient to deal first with the *Perameles* embryos, as a wax-plate reconstruction was made of the heart and anterior vessels of embryo A (Pl. II. figs. 6-8).

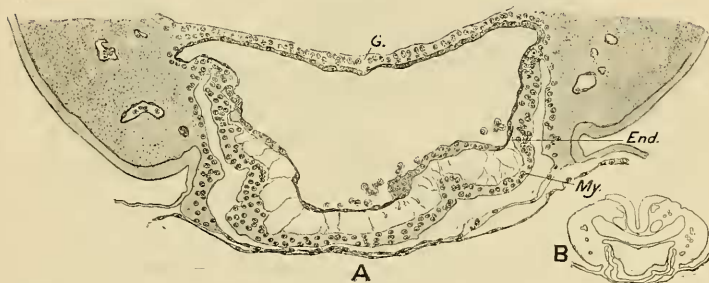
As regards general development, *Perameles obesula* (10. viii. 03) shows only a slight advance on *P. nasuta* (1 S) Stage III. The brain has practically not changed; the gut is in the same condition except that the first visceral pouch is more extensive and closure of the fore-gut has progressed back to slightly behind the auditory vesicle.

In the vascular system, however, we find a most marked advance, the heart having assumed a definite form with ventricular and auricular divisions recognisable. Figs. 6 to 8 illustrate the model of the heart and anterior vessels in this stage. The gut, included to form a building base, is coloured white, heart endothelium and arteries red, veins and most of the capillaries blue, myocardium yellow. The myocardium is left intact on the left half of the model from the roots of the aortic arches back to the level of the anterior end of the Cuvierian duct, but has been omitted on the right side so that the whole of the endothelial tube is here exposed to view. The capillaries surrounding the gut are also left intact on the left side of the model, but on the right have been omitted in order that the aortic arches might be seen more clearly. Study of the actual sections shows that the capillaries of the right side closely resemble those of the left.

The first and second visceral pouches are seen in the model as lateral projections from the gut (figs. 6-8, *V.P.* 1 & 2). The heart, which is median and ventral anteriorly, still consists of separate lateral primordia posteriorly, the two halves diverging in the lateral lips of the anterior intestinal portal (fig. 6, *A.I.P.*). By the great enlargement of the heart itself, the pericardial coelom has become relatively considerably reduced, and now simply forms a space surrounding the heart ventrally and ventro-laterally (text-fig. 18). The median pericardium extends from the cephalic extremity of the heart to the anterior intestinal portal.

At their cranial ends the endothelial tubes (exposed in the model by the omission of the myocardium) unite to form a broad conical portion, the bulbus (conus) arteriosus. The first and second aortic arches (*A.* 1 & 2) are given off from the dorsal side of the bulbus (fig. 8, *B.A.*, *A.* 1 & c.). In this region, the myocardium simply forms a continuous layer covering the endothelium,

Text-figure 16.

*Perameles obesula* (10.viii.03).

A. Transverse section through bulbus arteriosus.

B. Complete outline of same section.

End. Endothelium of bulbus arteriosus. *G.* Gut. *My.* Myocardium.

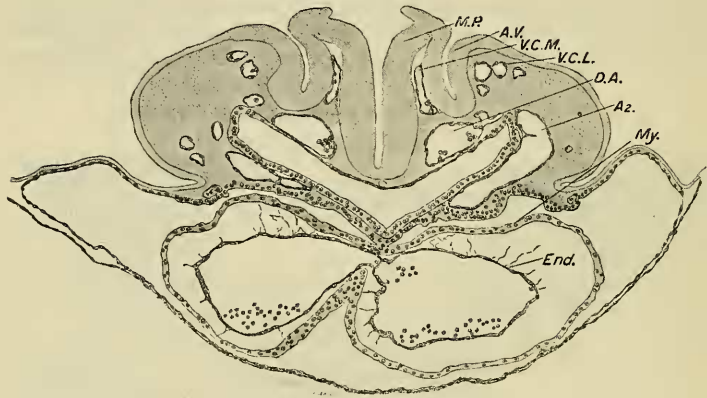
but separated from it by a space (text-fig. 16). The bulbus is the only portion of the heart in which the endothelial tubes have actually fused. Behind it, the tubes are in contact as far back as the anterior intestinal portal, but the wall between them is everywhere complete. The myocardium of the two sides has fused throughout the region of the closed gut and there is no ventral mesentery (text-figs. 16-18). There is, however, a very well marked groove on the ventral aspect of the myocardium, which marks the line of junction of the right and left halves. At the opening of the gut the right and left heart primordia separate completely.

From the ventral view of the model (Pl. II. fig. 6) it is obvious that already the heart has begun to bend between the two points (*a*) the roots of the aortic arches and (*b*) the opening of the fore-gut

The curvature, however, does not affect both sides equally, and a marked asymmetry results. The anterior ventricular portions of the heart-tubes are already being pushed backwards so as to lie ventral to the auricular portions. It is clear that by continuation of this curvature with accompanying fusion of the two halves, the typical embryonic relations of auricle and ventricle will ultimately be achieved.

In the ventricular region of the heart, the right and left endothelial tubes are approximately equal in size, but where there is an inequality the right is the larger (text-fig. 17, *End.*)

Text-figure 17.



Perameles obesula (10.viii.03). Transverse section through the ventricular region of the heart.

A₂. Second aortic arch. *A.V.* Auditory vesicle. *D.A.* Dorsal aorta. *End.* Endothelium. *M.P.* Medullary plate. *My.* Myocardium. *V.C.L.* Vena capitis lateralis. *V.C.M.* Vena capitis medialis.

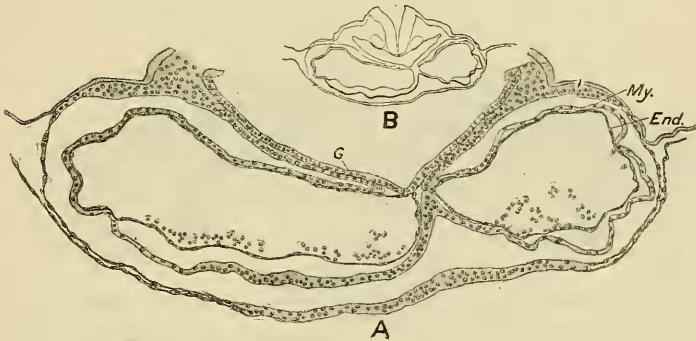
In addition to the curvature which is bringing the ventricular region into position ventral to the auricular region, there is a certain amount of curvature in the horizontal plane of the embryo.

In the right half of the heart, a definite constriction of the endothelial tube marks the limit between the ventricular and auricular portions. On the left side there is no such constriction. Posterior to this constriction the right endothelial tube widens out suddenly, reaching about three times its width in the constricted region. The left endothelial tube in this region widens only very slightly. The right and left heart primordia furthermore show considerable asymmetry as regards curvature, for while the portion of the left tube lying lateral to the open fore-gut is practically straight, the right tube in this region shows well marked

curvature. (See Pl. II. fig. 6.) Correlated with this difference in the size and curvature of the endothelial tubes, the opening of the fore-gut is also asymmetrical (fig. 6, *A.I.P.*). At the anterior intestinal portal, the right and left primordia of the heart separate, and both tubes become reduced in size, the right, however, more markedly than the left. The endothelial heart-tubes pass imperceptibly into vitelline veins.

A further distinction between the ventricular and auricular portions of the heart lies in the fact that in the anterior region the myocardium is separated from the endothelium by a considerable space crossed by fine strands of protoplasm (text-fig. 17), while in the posterior portion the myocardium is closely applied to the endothelium (text-fig. 18). The transition between these two conditions takes place gradually in the region of the atrio-ventricular constriction of the right side.

Text-figure 18.

*Perameles obesula* (10.viii.03).

- A. Transverse section through auricular portion of the heart.
 B. Complete outline of same section.

End. Endothelium. *G.* Gut. *My.* Myocardium.

Turning now to the blood-vessels, two aortic arches are complete. Their relations are seen most clearly in the side view of the model (Pl. II. fig. 8, *A. 1 & 2*). From the ventral view (fig. 6) it will be seen that there are a number of capillaries lying against the gut-wall, between the roots of the right and left mandibular arches. These are probably derived from the scattered angioblast cells in the corresponding position in Stage III., and are doubtless destined to contribute to the formation of the median ventral aorta which is established in the next stage (Stage VI.). The first aortic arch runs forwards to the anterior end of the gut and is there connected by a well developed loop, situated laterally to the apex of the fore-gut, with the corresponding dorsal aorta. The aorta is paired

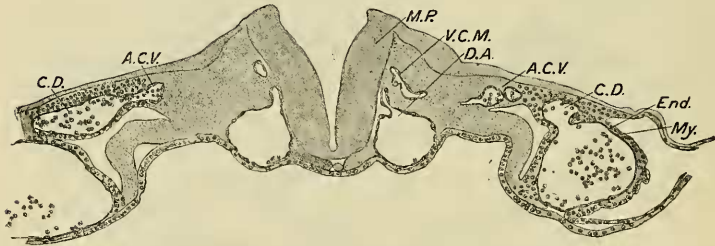
throughout its length (Pl. II. fig. 7, *D.A.*). From the anterior convexity of the loop of the first arch there is given off on each side a vessel which runs outwards and forwards and then breaks up into series of capillaries which form a cup surrounding the primary optic vesicles. These capillaries run round the posterolateral face of the optic vesicle to become connected dorsally with the veins of the head (fig. 7).

The second aortic arch arises from the dorsal side of the bulbus, runs backwards and outwards to loop round the gut in the hyoid arch and to join the dorsal aorta (see Pl. II. figs. 6-8, *A. 2*).

From the dorsal aortæ posterior to the second aortic arch, there arises a pair of small sprouts running outwards on the gut-wall. These are the dorsal elements of the third aortic arch.

The best developed venous trunk of the head, viz. the vena capitis medialis, is clearly seen in the dorsal view of the model (fig. 7, *V.C.M.*). Each is a small vein lying dorsal to the dorsal aorta

Text-figure 19.



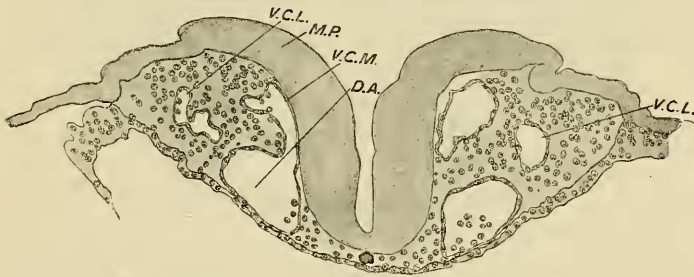
Perameles obesula (10.viii.03). Transverse section through the Cuvierian ducts.

A.C.V. Anterior cardinal vein. *C.D.* Cuvierian duct. *D.A.* Dorsal aorta. *End.* Endothelium. *M.P.* Medullary plate. *My.* Myocardium. *V.C.M.* Vena capitis medialis.

close against the neural tube (text-fig. 17, *V.C.M.*). Anteriorly this vein runs into the dorsal aorta. In the model this is only shown on the left side, but high-power examination of the sections reveals a very fine capillary completing the connection between the right vein and the aorta. Arising from the vena capitis medialis in the anterior half of the mandibular arch is a series of capillaries which are continuous with those surrounding the optic vesicles. The brain here is widely open and its margin is situated just to the outer edge of the capillaries. The latter would accordingly lie medial to the neural crest were such present in this region. In the region of the first visceral pouch there is another line of capillaries lying lateral to the vena capitis medialis and connected with it. From the anterior end of these capillaries there runs outwards and forwards a vessel connecting them with a group of capillaries lying in the mandibular mesoderm lateral

and ventral to the gut. The vena capitis medialis runs back alone for a short distance, and is then again connected with a more laterally situated capillary. This capillary runs back from this point to the level of the incipient third aortic arch, and then runs laterally to be connected with the Cuvierian duct which is now well developed (fig. 7 & text-fig. 19). On the right side of the embryo this transverse connection is very incomplete, but not quite so incomplete as would appear from the model, since the difficulty of building up these fine capillaries caused some to be lost in this region. This vein, lying lateral to the vena capitis medialis and to the primordia of the nerve-ganglia, is the vena capitis lateralis of Grosser (6) and Salzer (17). From the venæ capitis medialis and lateralis the anterior cardinal vein is derived. The auditory vesicle lies in the space between the venæ capitis medialis and lateralis immediately dorsal to the second aortic arch. Running in the hyoid arch are a few capillaries apparently corresponding with the much more conspicuous group in the mandibular arch.

Text-figure 20.



Perameles obesula (10.viii.03). Transverse section showing the connection of the vena capitis medialis with the dorsal aorta.

D.A. Dorsal aorta. *M.P.* Medullary plate. *V.C.M.* Vena capitis medialis.
V.C.L. Vena capitis lateralis.

The Cuvierian ducts have increased considerably in size, and that of the left side opens direct into the lateral heart-tube (text-fig. 19, *C.D.*). Behind the opening of the Cuvierian duct a single small capillary runs posteriorly in the somatopleure representing the future umbilical vein. The vena capitis medialis, it should be noted, continues on after the lateral bend of the vena capitis lateralis (fig. 7). The two are closely connected in the region of the incipient third aortic arch.

One important point which is difficult to observe in the figures of the model is shown in the sections of the embryo (text-fig. 20), and that is the fact that the vena capitis medialis at irregular intervals opens into the dorsal aorta.

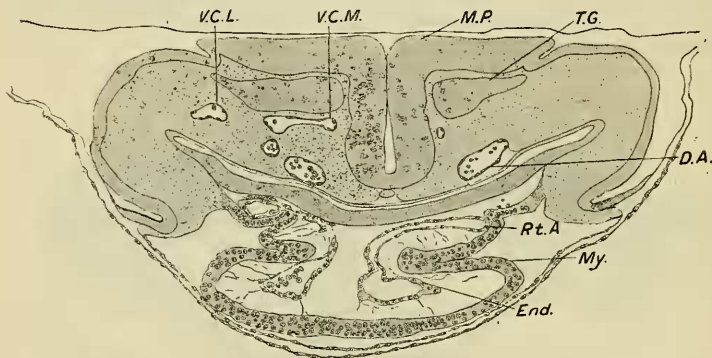
In this stage, then, we find the ventricular and auricular

portions of the heart differentiated. Fusion of right and left primordia has only affected the myocardium and the cephalic ends of the endothelial tubes. The two halves of the auricular portion of the heart are wide apart.

Two complete aortic arches are present and one is in process of formation. Venæ capitis medialis and lateralis and Cuvierian ducts are all present.

It is not necessary to give a detailed description of the embryo of *Macropus* sp., which is included in this stage, as it differs only in certain points from the *Perameles obesula* embryo described above. In the degree of development of the gut and pharyngeal pouches, as well as of the nervous system, the two embryos very closely resemble each other.

Text-figure 21.



Macropus sp. Transverse section through the root of the second aortic arch.

D.A. Dorsal aorta. End. Endothelium. M.P. Medullary plate. My. Myocardium.
Rt.A. Root of aortic arch. T.G. Primordium of trigeminal ganglion.
V.C.L. Vena capitis lateralis. V.C.M. Vena capitis medialis.

Two complete aortic arches are present, but there is no trace of a third.

The vena capitis medialis resembles that of the *Perameles* embryo. The vena capitis lateralis, however, is slightly less advanced, being only recognisable in the region of the trigeminal and facial neural crest proliferations, and not extending back as far as the auditory vesicle. Cuvierian ducts are present, and the right one at least opens into the heart-tube. The sections are somewhat broken in this region, so that satisfactory observations on the openings of the Cuvierian ducts and their relations to the anterior cardinal veins are impossible.

The heart differs in several respects from that of the *Perameles* embryo. The general relations of heart and pericardium and the mode of origin of the aortic arches are exactly the same in the

two embryos. In the *Macropus* embryo, as in *Perameles*, the myocardium is fused in the middle line throughout the length of the closed gut. A slight groove marks the line of fusion in the posterior portion of the heart, but there is no indication of a ventral mesentery at any point. The ventricular region of the heart is distinguished from the auricular by the fact that in the ventricular portion a considerable space intervenes between endothelium and myocardium, whereas in the auricular portion the two layers are close together. The limit between the two divisions is further indicated by a constriction of each endothelial tube, which then widens out abruptly to form the auricle. Right and left endothelial tubes are united anteriorly in the region of the bulbus arteriosus, just as in the *Perameles* embryo. Behind this again they diverge around the opening of the gut. It may be noted that in the region of the widest divergence of the endothelial tubes a fine bridge runs across and connects the two.

The curvature, so far as it can be made out without reconstruction, is similar to that of the *Perameles* embryo. The asymmetry appears to be less marked than in the latter embryo, but on this point it is impossible to make a positive statement without reconstruction. Throughout a considerable portion of its length, however, the right endothelial tube is larger than the left, just as in the *Perameles* embryo.

The *Macropus* embryo then differs from the *Perameles* embryo of a similar stage mainly in the configuration of the endothelial tubes, which are joined at their cephalic extremity, then widely separate for some distance (text-fig. 21), and then again approximated, though not joined. This difference, as well as the slight differences in the myocardial wall, may very probably be due to slight dissimilarity in the positions of the endothelial tubes and the myocardium before union of the latter.

STAGE VI. *Macropus ruficollis*.

The material for this stage consists of a single embryo of *Macropus ruficollis*, cut transversely.

Greatest length of embryo, 5.2 mm.

Dorsal perimeter, about 13.5 mm.

The embryo is sharply bent in front of the fore-limb buds, so that the head, invested by proamnion, is sunk down into the yolk-sac and forms an acute angle with the trunk. No trunk amnion is yet developed. The brain, though open in the fore- and mid-brains, is closed in the region of the hind-brain.

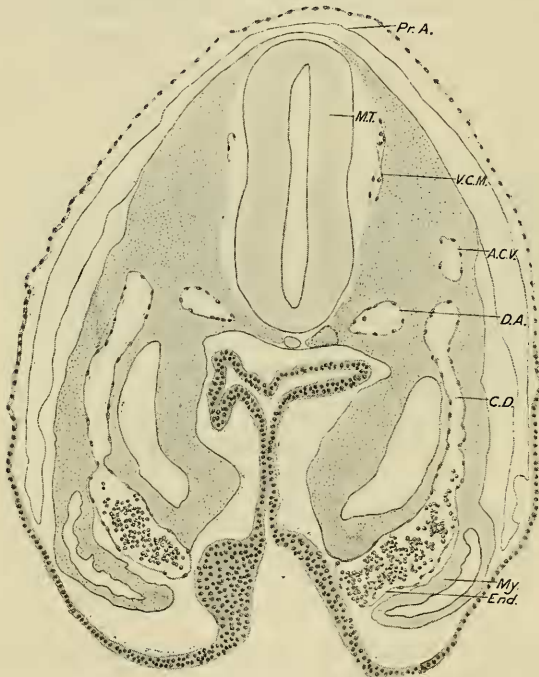
The gut is closed as far back as the third well-developed somite. Three visceral pouches are present.

The heart shows a considerable advance on the preceding stage. The right and left heart-tubes are fused except in the region of the sinus venosus, where they remain separate, while the Cuvierian

ducts, which are now established as wide vessels, pass across the coelomic cavity to open into the right and left heart-tubes (text-fig. 22).

Curvature of the median portion of the heart has resulted in the definite establishment of a U-shaped ventricular limb and an auricular portion extending from the left dorsal side of the ventricle posterior to its apex and separating, at the opening of the gut, into the right and left halves of the sinus venosus.

Text-figure 22.



Macropus ruficollis. Transverse section through the Cuvierian ducts.

A.C.V. Anterior cardinal vein. *C.D.* Cuvierian duct. *D.A.* Dorsal aorta. *End.* Endothelium. *M.T.* Medullary tube. *My.* Myocardium. *Pr.A.* Proamnion. *V.C.M.* Vena capitis medialis.

The cephalic portion of the S-shaped heart is somewhat curved, so that, as in the preceding stage, the bulbus (conus) arteriosus lies dorsal to the cephalic extremity of the ventricle. The bulbus arteriosus is continued into a short median ventral aorta which bifurcates to form the first pair of aortic arches. The second and

third pairs of aortic arches arise from the median ventral aorta immediately posterior to its bifurcation. The second arch is large, the third very small.

In correlation with the rapid development of the fore- and mid-brains at this stage, the head-plexus found in Stage V. (see Pl. II. figs. 6-8) has become extended into a long slender vessel, destined to form the anterior part of the internal carotid artery and lying on either side of the mid-ventral line in the fore-brain region. As in the preceding stage, it anastomoses anteriorly with capillaries arising from the vena capitis medialis.

Both venæ capitis medialis (text-fig. 22, *V.C.M.*) and lateralis are present, though neither can be traced continuously throughout the head-region. The vena capitis medialis extends to the anterior end of the brain, lying close to the medullary tube, dorsal to the dorsal aorta and the internal carotid artery. It is discontinuous in the region of the auditory vesicle, where no veins are recognisable. Posterior to that, it runs back as a continuous trunk to the level of the Cuvierian duct, and beyond this is recognisable as a minute vessel lying close to the neural tube in the trunk region.

Lying lateral to the primordium of the trigeminal nerve, there are a few scattered capillaries which represent discontinuous segments of the vena capitis lateralis. Immediately posterior to the primordium of the trigeminus, the vena capitis lateralis arises from the vena capitis medialis and runs back as a small vessel lying lateral to the root of the facial nerve. Venæ capitis lateralis and medialis are interrupted in the region of the auditory vesicle, but both are present immediately posterior to it. At irregular intervals on their course there are transverse communications between the two veins. The vena capitis lateralis does not form a continuous trunk in the region posterior to the auditory vesicle, but immediately anterior to the point of separation of right and left heart-tubes it increases markedly in size and is connected by a wide anastomosis with the vena capitis medialis, which becomes very small posterior to this level. The enlarged vena capitis lateralis, or, as it may here be called, anterior cardinal vein, passes ventro-laterally and, running alongside the dorsal aorta for a short distance, finally opens into the Cuvierian duct in the manner seen in text-fig. 22.

The umbilical vein is now present, running in the somatopleure and opening into the Cuvierian duct.

In this stage, then, we have a heart in which fusion of the right and left primordia has occurred except in the region of the sinus venosus, and curvature has carried the auricular limb into position dorsal to the ventricle. Three aortic arches are present, venæ capitis medialis and lateralis are well established though discontinuous and open *viâ* the Cuvierian ducts into the sinus venosus.

SUMMARY AND DISCUSSION.

A. *Development of the Heart.*

From the foregoing description it is evident that the early development of the heart in such Marsupials as *Perameles* and *Dasyurus* proceeds along essentially the same lines as in Eutheria. The early stages of the heart development in the latter have been described by a number of investigators (*e.g.* Mollier (15)); but although the broad outline of the process may be said to be well known, there is still considerable difference of opinion with regard to certain points. It will be useful, therefore, before summarising the preceding observations, to briefly review the literature on the subject.

With regard to the lateral paired primordia of the heart little need be said at this point. The heart endothelium arises between the entoderm and the splanchnic mesoderm, from which latter it is either partially or wholly derived. The primordia of the heart-tubes are first recognisable in the hind-brain region and grow forwards at the expense of angioblastic cells proliferated off from the splanchnic mesoderm, which is itself thickened and indented to form the primordium of the myocardium. It should be noted that in the earliest stages examined (Pl. I. figs. 1 & 2) the endothelium lies to the medial side of the pleuro-pericardial canals in the anterior region and to the lateral side in the posterior region. To this point reference will be made subsequently in connection with the discussion of the reversal of the pericardium which, according to some authors, takes place at the time of formation of the head-fold.

The processes which bring the lateral heart primordia into position below the fore-gut relate primarily to the formation of the head-fold, and it is therefore necessary to get a clear idea of the mode of closure of the gut before considering the problems relating to the fusion of the lateral primordia of the heart.

Some authors (*e.g.* Robinson (13)) hold that the formation of the fore-gut is due mainly, if not entirely, to the rapid growth of the embryo over the relatively stationary line between the embryonal and extra-embryonal areas. Thus Robinson (13) says: "The orifice (of the umbilicus) is not reduced in size during the early stages of development by the convergence of its margins towards a central point. This being the case, no tucking off of the embryo from the surface of the ovum can occur; on the contrary, what does occur is almost the exact opposite of such a process, for the margin of the area remains as a relatively slow-growing region, whilst the embryonic and extra-embryonic portions of the wall of the ovum rapidly increase in extent. Under these circumstances, it follows that the margin of the embryonic area will soon appear as a ring between the upper or embryonic and the lower or extra-embryonic parts of the ovum, both of which have expanded beyond it in all directions."

While the forward growth of the brain-plate doubtless plays an important part in the initiation of the formation of the fore-gut, this explanation does not account for the conditions revealed by reconstructions of the gut and pericardium in the early stages of head-fold formation.

On the other hand, various investigators (*e. g.* Rouvière (14), Gräper (4)) contend that there occurs, in addition to the forward growth of the brain-plate, a backward progression of the anterior intestinal portal, whilst in older accounts an actual fusion of lateral folds in the mid-ventral line was assumed. Both Robinson (13) and Rouvière (14) give excellent reasons for regarding this assumption as erroneous. They point out that if gut-closure were effected by the fusion of lateral folds (such as are shown in text-fig. 9), the heart would remain in connection with the gut by a dorsal mesocardium and with the yolk-sac wall by a ventral mesocardium. Robinson denies the existence of a ventral mesocardium in mammals, and quotes this fact in support of his theory that the separation of the gut from the yolk-sac is due to growth of the embryo rather than to fold-formation. Rouvière, on the other hand, while he agrees with Robinson as to the absence of a ventral mesocardium in mammals, gives a different account of the process of gut-closure. He describes the formation of lateral pleuro-pericardial canals which grow forwards round the anterior end of the brain-plate and fuse to form a continuous cavity. The splanchnopleure forming the posterior wall of the pleuro-pericardial cavity now forms a continuous fold which Rouvière, following Tourneux, calls the "cardiac fold" (compare text-fig. 12, *C.F.*) and which he describes as growing actively backwards as a whole.

In the chick, on the other hand, a ventral mesocardium is present, but this is due, as Robinson points out, to the relatively late penetration of the mesoderm in the head region. The pleuro-pericardial canals do not extend round and unite in front of the medullary plate in early stages, but only at a later stage do they penetrate into the floor of the fore-gut after that has been formed. The lateral cavities therefore do not at once become continuous, but remain separated from each other by a double layer of mesoderm constituting the ventral mesocardium.

With regard to mammals, Rouvière, while he does not discuss the influence of the forward growth of the brain-plate, concludes that the crescent-shaped cardiac fold grows backwards as a whole, and that the free edge of the splanchnopleural fold progresses always in advance of the primordia of the heart, so that no fusion of the splanchnopleure is involved and no ventral mesocardium is formed.

Gräper, in a description of the growth processes in the developing chick, which he worked out by staining the living embryos and keeping them under observation while still alive, shows that there is considerable evidence in support of the view that the margin of the fore-gut (anterior intestinal portal) moves

backward concurrently with the forward growth of the brain-plate. He gives a series of comparative measurements which show that the rate of removal of the lip of the anterior intestinal portal from the anterior end of the brain is greater than the rate at which the brain-plate grows forward from a given fixed point; hence it is evident that the anterior intestinal portal must be moving backwards.

Concurrently with the formation of the fore-gut, the lateral heart-tubes come to lie ventrally to it, but do not at once fuse. Wilson (20), in a paper on young human embryos, draws attention to this fact and refers to the embryo of *Perameles nasuta*, described in this paper as Stage III., as exemplifying this condition; but he does not discuss the question as to how these lateral hearts approach one another.

We may now consider the evidence afforded by the material described above, and will endeavour to show that it is entirely in accord with the view that there is actual backward growth of the anterior intestinal portal, and that it is this process, and not fusion of lateral folds, that brings about lengthening of the fore-gut.

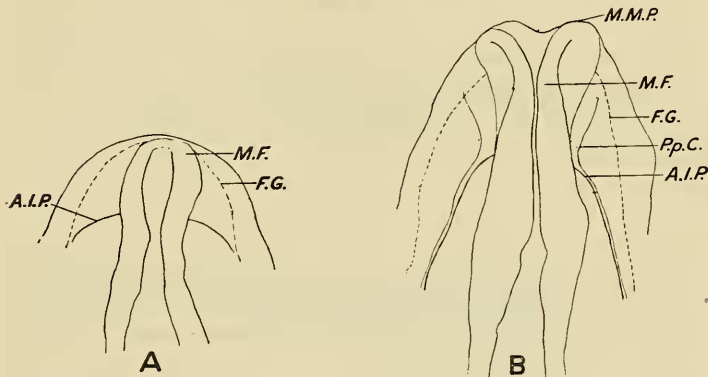
It may be noted here that in *Perameles*, as in the rabbit (Rouvière), no ventral mesocardium is present at any stage, a fact which, in itself, is a strong argument against the theory that gut-closure is effected by the fusion of lateral folds.

If we compare a stage in which the head-fold has not yet appeared (Pl. I. figs. 1 & 2) with one in which a small portion of the fore-gut is differentiated (figs. 3 & 4), we see that the pleuro-pericardial celom not only moves backward relatively to the brain-plate but also increases very considerably in width. It is obvious that such an increase in size must either cause the pericardium to extend peripherally or to close in towards the axial line, and it is perfectly clear on comparison of figs. 2 & 3 that it is this latter process which is taking place. From a longitudinal section, such as is shown in text-fig. 4, it is evident, moreover, that such an expansion of the pericardium must involve the backward growth of the splanchnopleural floor of the fore-gut. If the lengthening of the fore-gut were due entirely to the rapid forward growth of the brain-plate, there would be no such inward closure of the pericardial region. Moreover, if we compare figs. 2 & 3 (Pl. I.), we see that in the earlier stage, the pericardial celom is situated peripherally to the margin of the brain-plate, while in the second stage, the inner margin of the pericardium lies in the lip of the anterior intestinal portal. Now the growth in length of the brain-plate in the period between these two stages would naturally give rise to a fold round its anterior margin, but would not bring the pericardium into the position it occupies in Stage II. (fig. 3), unless there occurred concurrently with such growth in length either an increase in width of the brain or an inward closure of the pericardium. Comparison of figs. 2 & 3 again shows that while no increase in width of the

brain-plate has occurred, the pericardium has actually closed in towards the axial line. Precisely the same conclusion may be reached from a comparison of figs. 1 and 4, but as the interval between the stages is greater and the embryos are not of the same species, less importance attaches to them in this connection.

It has already been remarked that, in the chick, the development of the pleuro-pericardial canals occurs at a later period than in the mammal, so that in this type the form of the head-fold in early stages cannot be affected by growth of the pericardium. If we compare the shape of the fore-gut in a chick of two somites with that of *Dasyurus* Stage II. (text-fig. 23 A & Pl. I. fig. 3), we see that in the first stage of head-fold formation in the bird, the outline of the anterior intestinal portal is broadly

Text-figure 23.



Anterior end of chick of (A) 2 somites, (B) 4 somites, to show the relations of the head-fold, brain-plate, anterior intestinal portal and pleuro-pericardial cavities.

A.I.P. Anterior intestinal portal. F.G. Fore-gut (outline). M.F. Medullary fold.
M.M.P. Margin of the medullary plate. P.p.C. Pleuro-pericardial cavity.

crenescentic, while in *Dasyurus* it is U-shaped. This difference I conceive to be due to the fact that in the chick, no factor but the forward growth of the brain-plate is operating at this stage, while in the mammal, in addition to this process, the expansion of the pericardium is already bringing about the formation of lateral folds and the consequent narrowing of the anterior intestinal portal. A slightly later stage of the chick (text-fig. 23 B) shows an approximation to the mammalian condition, for the pleuro-pericardial canals have appeared and are progressing towards the middle line; lateral folds have therefore arisen and the outline of the anterior intestinal portal is U-shaped.

We may therefore conclude, that while the forward growth of

the brain-plate initiates the formation of the head-fold, there occurs concurrently with this process in the mammal, and at a slightly later stage in the chick, a rapid expansion of the pericardium and a consequent backward and inward growth of the fold of splanchnopleure which constitutes the inner margin of the pleuro-pericardial cavity.

Additional evidence is afforded by the study of the longitudinal sections and reconstructions of Stages III. & IV. (text-figs. 12 & 15; Pl. I. figs. 4 & 5). From the longitudinal sections, it is evident that a great increase in length of the brain has occurred in the mid- and fore-brain regions. If, therefore, the increase in length of the fore-gut were due to elongation of the brain-plate, a corresponding increase should occur in the portion of the fore-gut lying below these segments, *i. e.* the portion anterior to the first visceral pouch in Stage III. (fig. 4). Comparative measurements of the gut in figs. 4 & 5 show, however, that no increase in length has occurred anterior to the first visceral pouch. Moreover, growth of the medullary plate would not necessarily bring about lengthening of the fore-gut unless it occurred along a straight line representing the longitudinal axis of Stage III., *i. e.* unless the brain remained unflexed. The positions of the auditory neuro-mere in fig. 4 (opposite the second visceral pouch) and fig. 5 (opposite the first visceral pouch), show that the brain-plate has moved forward relatively to the gut between Stages III. & IV. If, now, we study the longitudinal section of Stage IV. (text-fig. 15) we see the conditions resulting from the increase in length and forward growth of the brain-plate. The gut has increased in dorsi-ventral extent, the medullary plate projects considerably anterior to the cephalic limit of the gut, and flexure has occurred at two points; that is to say, the rapid growth of the fore- and mid-brains, instead of involving a longitudinal stretching of the portion of the gut lying ventral to them, has caused little or no increase in length of the embryo along its straight long axis: the additional extent of the brain-plate is accommodated within the limited space by flexure.

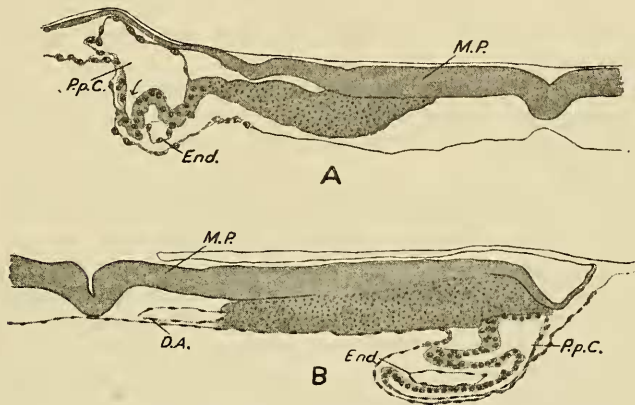
We see, therefore, that although the brain-plate lengthens rapidly after the first establishment of the head-fold, we can safely conclude that this does not cause elongation of the gut, for the regions of greatest growth of the gut and brain-plate are not correlated and the value of the forward growth of the brain as a factor in the lengthening of the fore-gut is largely rendered nugatory by the occurrence at this period of the cranial flexure.

On the other hand, there is little difficulty in interpreting the progressive closure of the gut at this stage as being due to an entirely different cause, for the median pericardium has extended rapidly, its antero-posterior length in the middle line having more than doubled in the short developmental period elapsing between Stages III. and IV. (Pl. I. figs. 4 & 5). As the anterior margin of the pericardium is in contact with the ectoderm of the head-fold, the rapid expansion of the cavity naturally involves a

closure inwards, towards the axial line, of the fold of splanchnopleure limiting the gut.

It is necessary now to consider the early development of the heart in relation to the mode of closure of the gut described above. It is evident, as Rouvière points out, that if gut-closure be effected by the backward movement of the cardiac fold, no ventral mesocardium will be formed at any stage in forms, such as mammals, in which a continuous pericardial cavity is present prior to head-fold formation. It remains to be considered how the lateral primordia of the heart reach their position in the dorsal wall of the median pericardium. Various authors, *e. g.* Robinson (13), have assumed that as the head-fold forms, the pericardium undergoes a complete reversal, so that its anterior

Text-figure 24.



Transverse sections through embryos of *Dasyurus viverrinus*, (A) Stage I. (7.5 mm.), (B) Stage II. (8.5 mm.), to show the direction of extension of the pericardial cavity.

D.A. Dorsal aorta. *End.* Endothelium. *M.P.* Medullary plate.
P.p.C. Pleuro-pericardial canal.

wall becomes posterior and its ventral wall, dorsal. Of such a process of reversal, the longitudinal sections figured here (text-figs. 4, 5 & 12) give no evidence. Moreover, in the anterior region of the pericardium, the primordia of the heart on each side of the embryo lies at or near the inner, medial margin of its pleuro-pericardial canal, so that a reversal which affected the anterior limb of the pericardium would indeed bring the heart primordia into position ventral to the gut, but would carry them also to the lateral margins of the gut, a position which they do not occupy.

On the other hand, if we take into consideration the fact that

the heart primordia lie at the medial margin of the pleuro-pericardial canals, we see that the inward progression of the edge of the splanchnopleural fold in the direction indicated by an arrow in text-fig. 24 A, will bring about the conditions shown in text-fig. 24 B. (Compare also text-fig. 8, where the relations are essentially the same and probably approximate more closely to those in the living embryo.)

From this stage, it is evident that when the lateral limbs of the pleuro-pericardial canals become incorporated in the median pericardium by the backgrowth of the cardiac fold, the heart primordia will lie in the dorsal wall of the pericardium and will be situated towards the middle line of the gut. (See text-fig. 8.) Thus, as Rouvière (14) indicates in describing similar conditions in the rabbit, the position of the heart primordia in such a stage as is represented in text-fig. 24 B, is brought about "by the inward extension of the lateral prolongations of the cardiac fold." There is no evidence of reversal of the pericardium, nor is there adequate ground for assuming that such a process occurs.

We can, therefore, gain a clear conception of the way in which the lateral heart primordia attain the position they occupy in Stage III. (Pl. I. fig. 4; text-fig. 8) lying side by side below the closed fore-gut.

In order to complete the history of the early development of the heart, it is now necessary to consider the mechanism which brings the heart-tubes into contact in the middle line.

If we compare figs. 4 and 5 (Pl. I.) we see at once that while the pericardium has increased rapidly in the antero-posterior direction, it has not increased in transverse width and, in fact, at the point of closest approximation of the heart-tubes, an actual decrease in width has occurred; that is to say, the pericardium at this stage is growing in the antero-posterior direction at the expense of its transverse width. This fact suggests a simple explanation of the approximation of the heart-tubes after gut-closure, for it may be supposed that if the total width of the pericardium is reduced by this process of stretching, the distance between the heart-tubes will decrease until they meet each other in the middle line*.

The heart-tubes, in the period following immediately on their reaching the middle line, grow very rapidly, so that, in the next stage (V.), we find various forms of curvature which serve to accommodate the increased length of the heart. In the embryo of *Perameles obesula* described in this stage (Pl. II. fig. 6), the heart-tubes are in contact through a great portion of their length and here follow a parallel curved course. The separate heart-tubes lying in the lips of the anterior intestinal portal, however, show a marked difference from each other both in their length and the

* The suggestion that the approximation of the heart-tubes is due to such a growth in length without compensatory growth in width was made to me by Professor Hill.

form of curvature, the right primordium being larger and more markedly curved than the left. This asymmetry occurs to a somewhat less extent in the *Onychogale* embryo of this stage, and also in a number of embryos of *Dasyurus viverrinus* of about the same stage, so that evidently at this period the right and left primordia of the heart develop independently of each other. It may be suggested that the greater length of the right heart-tube is to be accounted for by the fact that it is destined to form the convex, longer side of the completed ventricular limb.

B. *Development of the Cardinal Veins.*

Before summarising the results of my observations on the development of the cardinal veins, more particularly the anterior cardinals, it may be useful to give a short resumé of previous work in this field.

Hoffmann (7) in 1893 described the development of both anterior and posterior cardinals in Selachians by the formation of a series of offshoots from the dorsal aortæ. These offshoots become connected on each side to form a continuous longitudinal trunk. He figures capillaries lying on both medial and lateral sides of the auditory vesicle, but makes no comment thereon.

Salzer (17) in 1895 described the development of the anterior cardinal veins in the guinea-pig. According to him, the first vein of the head arises on the medial side of the cranial ganglionic primordia. A vein next arises lying lateral to the ganglionic primordia of nerves VII., IX. & X. and to the auditory vesicle. This vein, which Salzer calls "*vena capitis lateralis*," communicates with the medial vessel and seems to be formed from a series of lateral outgrowths from it. The medial vessel degenerates in the region of nerves VII. to X., so that for a time there is a condition in which the vein of the head runs medially to the trigeminal nerve, then, passing laterally, runs outside nerves VII., IX. & X. and the auditory vesicle, and finally passes round the medial side of nerve XII. before opening into the Cuvierian duct. In subsequent stages, the process of development of the lateral trunk is continued anteriorly and posteriorly in the region of the trigeminal and hypoglossal. Thus the definitive anterior cardinal vein runs laterally to all the cranial nerve-roots.

Grosser (6) in 1907 gave a similar description of the development of the anterior cardinals throughout the vertebrate series. He calls the vein lying medial to the nerve-roots the *vena capitis medialis*, and the lateral vessel the *vena capitis lateralis*. The former develops first and lies close against the neural tube. From it are given off lateral vessels which become connected on the outer side of the nerve-roots to form the *vena capitis lateralis*. The *vena capitis medialis* persists only at its anterior end, the rest of the anterior cardinal being derived from the *vena capitis lateralis*.

Turning now to the facts revealed by the foregoing study of *Perameles*, we find both the venæ capitis medialis and lateralis present. In Stage III. the first traces of the venous system of the head are present in the form of isolated segments of the vena capitis medialis. Further, in this stage, in the region of the somites, there is on each side a series of dorsal offshoots from the dorsal aorta (text-fig. 11) partially connected to form an as yet incomplete longitudinal vessel lying, like the vena capitis medialis, close against the neural tube. This vessel Hoffmann (7) described as representing the primordium of the posterior cardinal vein. It is worthy of note that whilst the origin of this vein from the dorsal aorta is thus clearly demonstrated, no connection between the anterior segments of the vena capitis medialis and the dorsal aorta could be observed, even after careful study of the individual sections under the high power. In the next stage (IV. *P. nasuta* 2 P), however, the vena capitis medialis, though not forming a continuous longitudinal trunk, is recognisable throughout the head region and is connected at irregular intervals with the dorsal aorta. Furthermore, the vena capitis medialis in this stage gives off lateral capillaries which anastomose to form the primordium of the vena capitis lateralis. In the somitic region we find again a series of inter-segmental offshoots from the dorsal aorta. The venæ capitis lateralis and medialis continue to develop side by side, giving rise to the condition shown in Pl. II. fig. 7 (*V.C.L.* and *V.C.M.*). (See also text-fig. 25.) In this stage the dorsal aorta and the vena capitis medialis are connected by small capillaries (see text-fig. 20) whilst anteriorly the two vessels pass into continuity with each other*. From the material available it is not possible to say definitely how these connections arise, but the facts suggest that the vena capitis medialis is derived from the dorsal aorta. This view is further supported by the existence in the trunk region of a longitudinal vessel which is undoubtedly formed from a series of outgrowths from the dorsal aorta (text-fig. 11). This vessel apparently bears the same relation to the posterior cardinal that the vena capitis medialis does to the anterior cardinals, *i. e.* it gives origin to capillaries which contribute to the formation of the posterior cardinal. The origin of the vena capitis medialis from the dorsal aorta cannot, however, be regarded as proved, for in the first stage in which it is recognisable, no connection with the dorsal aorta could be traced; in the two following stages (IV. and V.) the connection is established and is lost in all subsequent stages (*e. g.* VI.).

From the descriptions of Salzer (17) and Grosser (6) it seems that in the forms which they have studied, the vena capitis medialis fuses in its entirety with the vena capitis lateralis, and

* Professor Hatta tells me that he has found this condition also in the embryo of the lamprey.

the anterior cardinal vein formed by the fusion of these two vessels passes ventro-laterally to open into the Cuvierian duct. This description is not, however, applicable to *Perameles*. In Stage V. (Pl. II. fig. 7) the vena capitis medialis is continued backwards into the trunk region of the embryo. The vena capitis lateralis lies parallel with it and communicates with it repeatedly in the anterior region; it then diverges from it and constitutes here the vessel usually known as the anterior cardinal vein, opening into the Cuvierian duct. The posterior prolongation of the vena capitis medialis continues as a small vessel lying alongside

Text-figure 25.

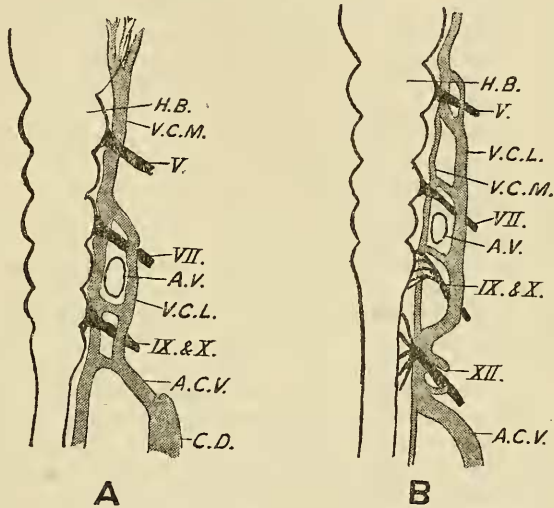


Diagram of the relations of the venae capitis medialis and lateralis to the primordia of the nerves. Viewed from the dorsal aspect.

A. Stage V. *Perameles obesula* (10.viii.03). B. Stage with twelve cranial nerves, *Perameles nasuta* (13.vii.05).

A.C.V. Anterior cardinal vein. A.V. Auditory vesicle. C.D. Cuvierian duct. H.B. Hind-brain. V.C.L. Vena capitis lateralis. V.C.M. Vena capitis medialis. V. Trigeminal nerve. VII. Facial nerve. IX. & X. Common root of glosso-pharyngeal and vagus nerves. XII. Hypoglossal nerve.

the neural tube in the trunk region. The Cuvierian duct arises as a relatively large vessel lying in the somatopleure dorsal to the posterior portion of the lateral heart-tubes. From its posterior extremity there runs back a series of capillaries which anastomose with capillaries arising from the prolongation of the vena capitis medialis. From this line of capillaries, which thus

shows a double origin, the posterior cardinal vein is undoubtedly derived, but the details of the process of development of the postcardinals cannot satisfactorily be worked out in the material available. The above account, however, agrees with that of Evans (2) for the chick with regard to the origin of the postcardinal from capillaries derived partly from the Cuvierian duct and partly from a vessel lying close to the neural tube.

The vena capitis lateralis in Stage V. is connected anteriorly with the primary head capillaries arising from the first aortic arch and also with groups of capillaries in the mandibular and hyoid arches (see Pl. II. figs. 6-8).

In subsequent stages, the development follows the course described by Salzer (17) and by Grosser (5). Thus in a stage in which twelve cranial nerves are established (*Perameles nasuta*, 13. vii. 05), the anterior cardinal vein runs medial to nerves V. and XII. and lateral to VII., IX., and X., and to the auditory vesicle (text-fig. 25 B); *i. e.*, the portion in the region of and anterior to the trigeminal nerve and also that posterior to the vagus, is derived from the original vena capitis medialis, the intervening portion from the vena capitis lateralis. Traces of the vena capitis medialis are, however, still present on the medial side of nerves VII., IX., and X.

Florence Sabin (16), in a recent note on the development of cardinal veins in the chick, supports the view that the cardinal veins are derived from the dorsal aorta. She, however, states: "The part of the head vein which lies close to the neural tube arises from the arch of the aorta and is a part of the vascular system of the central nervous system; the caudal part of the head vein arises directly from the aorta." In this respect my results differ somewhat from hers, for in *Perameles* the vena capitis medialis (*i. e.* "the part of the head vein which lies close to the neural tube") is present before there is any trace of the capillaries arising from the arch of the aorta (Stage III.). It is indeed secondarily connected with these, but as is shown in Pl. II. fig. 7 (*V.C.M.*) it also extends up to the extreme anterior end of the head in close relation to the dorsal aorta with which, in fact, it fuses. Since this vein exists before the formation of the head capillaries which connect it with the arch of the aorta, it obviously cannot be derived from that arch.

In seems, therefore, that in Selachians (Hoffmann (7)), the chick (Evans (2), Florence Sabin (16)), and also in *Perameles* and *Macropus*, there exists in the primary condition a continuous vessel lying close to the nerve-cord throughout its length. This vessel is derived in the posterior and probably also in the anterior region from the dorsal aorta. It contributes to the formation of both anterior and posterior cardinal veins.

It may be concluded that the presence in early stages of a vein lying close against the neural tube throughout its length is correlated with the relatively great importance of the central

nervous system in these stages. The brain and spinal cord, being the first organs to attain any considerable degree of development, are naturally the first to receive a vascular supply, and both *venæ capitis lateralis* and *medialis* persist for some time, forming a rich supply of capillaries to the brain and surrounding the developing cranial nerves.

FINAL SUMMARY AND CONCLUSIONS.

The facts revealed by the study of early stages in the development of Marsupials point to the conclusion that while the initiation of head-fold formation is in all probability due to the forward growth of the brain-plate, there occurs also an active backward growth of the anterior intestinal portal. This process is associated with the rapid expansion of the pericardium which occurs at this period of development, and which brings about the backward and inward growth of the layer of splanchnopleure limiting the pericardium.

In the course of this inward closure, the pericardial cavity extends to the ventro-lateral and finally to the ventral side of the lateral primordia of the heart, so that when the lateral portions of the pericardium become incorporated in its median limb, the heart primordia lie in the dorsal wall of the pericardium.

The approximation of the heart-tubes after gut-closure is due to the fact that, at this period, the pericardium grows rapidly in length and decreases in width so that the heart-tubes are brought together by longitudinal stretching of the pericardial wall lying between them.

Curvature of the heart is due to its rapid growth at a period of less active extension of the pericardium.

The first two aortic arches in *Perameles* are typical, and the development of the veins of the head resembles that process in other mammals in that the anterior cardinal vein is derived from persistent portions of two primitive head-veins, the *venæ capitis medialis* and *lateralis*. The posterior continuation of the *vena capitis medialis* also contributes to the formation of the posterior cardinal vein and is itself derived from the dorsal aorta.

REFERENCES TO LITERATURE.

1. BREMER, J. L.—“The Development of the Aorta and Aortic Arches in Rabbits.” *American Journal of Anatomy*, vol. xxx.
2. EVANS, H. M.—“On the Development of the Aortæ, Cardinal and Umbilical Veins and other blood-vessels of Vertebrate Embryos from Capillaries.” *Anatomical Record*, vol. iii.
3. EVANS, H. M.—Development of the Vascular System, in Keibel and Mall's ‘Text-book of Human Embryology.’

4. GRÄPER, L.—“Beobachtung von Wachstumsvorgängen an Reihenaufnahmen lebender Hühnerembryonen nebst Bemerkungen über vitale Färbung.” Archiv für Entwicklungsmechanik der Organismen, Bd. xxxiii.
5. GROSSER, O. & BREZINA, E.—“Ueber die Entwicklung der Venen des Kopfes und des Halses bei Reptilien.” Morph. Jahrb. Bd. xxxiii., 1895.
6. GROSSER, O.—“Die Elemente des Kopfvenensystems der Wirbeltiere.” Verh. d. Anat. Ges. Erg.-Heft. z. Anat. Anz. Bd. xxx., 1907.
7. HOFFMANN, C. K.—“Zur Entwicklungsgeschichte des Venensystems bei den Selachiern.” Morph. Jahrb. Bd. xx., 1893.
8. HOCHSTETTER.—“Die Entwicklung des Blutgefäßsystems.” Hertwig's Handbuch d. vergl. u. exper. Entwicklungslehre d. Wirbeltiere, Bd. ii. 2, 3.
9. KEISER, W.—“Untersuchungen über die erste Anlage des Herzens, der beiden Längsgefäßstämme und des Blutes bei Embryonen von *Petromyzon planeri*.” Jena. Zeitschr. für Naturw., vol. lvii.
10. LEWIS, F. T.—“The Intra-embryonic Blood-vessels of Rabbits from 8½ to 13 days.” Amer. Journ. Anat. vol. iii.
11. MALL, F. P.—“On the Development of the Blood-vessels of the Brain in the Human Embryo.” Amer. Journ. Anat. vol. iv.
12. MILLER & McWHORTER.—“Experiments on the Development of Blood-vessels in the area pellucida and embryonic body of the Chick.” Anat. Record, April 1914.
13. ROBINSON, A.—“The Early Stages of Development of the Pericardium.” Journal of Anatomy and Physiology, vol. xxxvii., 1902.
14. ROUVIÈRE, H.—“Études sur le développement du péricarde chez le lapin.” Journal de l'Anatomie, vol. xl., 1904.
15. RÜCKERT & MOLLIER.—“Die erste Entstehung der Gefäße und des Blutes bei Wirbeltieren.” Handb. d. vergl. u. exper. Entwicklungslehre d. Wirbeltiere, herausg. von O. Hertwig, Bd. i.
16. SABIN, F. R.—“On the Origin of the Duct of Cuvier and the Cardinal Veins.” Proceedings of Amer. Assoc. of Anatomists, Anat. Record, vol. ix. No. 1, 1915.
17. SALZER, H.—“Ueber die Entwicklung der Kopfvenen des Meerschweinchens.” Morph. Jahrb. Bd. xxiii., 1895.
18. SCHULTE, H. VON W.—“Early Stages of Vasculogenesis in the Cat with especial reference to the mesenchymal origin of endothelium.” Memoirs of the Wistar Institute of Anatomy and Biology, No. 3, 1914.
19. TANDLER, J.—“Zur Entwicklungsgeschichte der Kopfarterien bei den Mammalia.” Morph. Jahrb. Bd. xxx., 1902.
20. WILSON, J. T.—“Observations upon Young Human Embryos.” Journal of Anatomy & Physiology, vol. xlvi., 1914.